

association reactions. However, understanding the structure and dynamics of macromolecules in a cell is complicated by the highly crowded nature of the cell. It is likely that properties of macromolecules in cell may differ significantly to that measured in dilute solution. Diffusion plays important roles in many processes occurring inside the cell. The estimation of diffusion coefficient of macromolecules in a cell can be considered as a first step in understanding the complex nature of the heterogeneous environment of the cell.

In this current work we developed a computational model of *E. coli* cytoplasm and performed extensive Brownian dynamics simulation to calculate diffusivity of proteins. Our model differs from some of the previous models of *E. coli* cytoplasm in the following way; (1) The proteins modeled as flexible units by considering them as a collection of spheres. (2) hydrodynamic interaction (HI), which is essential to get accurate diffusion coefficient, was considered using a mean field approach.

The model predicts accurately the diffusion coefficient of Green Fluorescent Protein (GFP) in *E. coli* cell. We have found that HI is essential to get correct diffusion coefficient for this highly crowded system. The presence of anomalous diffusion has also been observed for short time (~1 micro sec), which was identified using fractional Brownian motion (FBM) analysis. It was found that repulsive interaction between different proteins is the main reason for the anomalous diffusion. To understand the anomalous diffusion observed in simulations, we also formulated a one dimensional random walk model in which successive steps are biased and correlated. This analytical model can explain some of the findings from our simulation.

## Voltage-gated K Channels I

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#### Movements of the Kv2.1 and Kv6.4 S4 Segments in Heterotetrameric Kv2.1/Kv6.4 Channels

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The voltage-gated K<sup>+</sup> (Kv) channel subunit Kv6.4 does not form functional channels on its own but tetramerizes with Kv2.1 subunits into functional Kv2.1/Kv6.4 heterotetramers with a proposed 3:1 stoichiometry. Within these Kv2.1/Kv6.4 heterotetramers, Kv6.4 causes an approximately 40 mV hyperpolarizing shift in the voltage-dependence of inactivation as compared to Kv2.1 homotetramers without affecting the voltage-dependence of activation significantly. By comparing the gating current (IQ) recordings of homotetrameric Kv2.1 and heterotetrameric Kv2.1/Kv6.4 channels, we recently showed that a second component in the charge (Q) versus voltage (V) distribution appeared in heterotetramers. Since this component develops at more negative potentials than Kv2.1 homotetramers, these results suggest that the voltage sensor of Kv6.4 subunits move in a more negative voltage range than the remaining Kv2.1's voltage sensors. Using cysteine accessibility studies, we show here that the voltage dependence of the rates of MTSET modification at V335C in Kv6.4 correspond with the second component of the QV distribution of Kv2.1/Kv6.4 heterotetramers. Similarly, the voltage dependence of modification rates at V296C in Kv2.1 follow the QV distribution of Kv2.1 homotetramers. These results indicate that in functional Kv2.1/Kv6.4 heterotetramers voltage sensors from Kv6.4 subunits move at more negative potentials than voltage sensors belonging to Kv2.1 subunits. (Supported by a FWO post-doctoral fellowship and FWO travel grant to EB and the Intramural Section Program of the National Institute of Neurological Disorders and Stroke, National Institutes of Health to MH)

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#### Ion Channels and Salt Bridges: Quantum Calculations Show Unusual Effects

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Calculations on voltage sensing domains (VSD) of Kv1.2 (pdb: 2A79/3Lut), with a mutation in which cysteine replaces an S4 arginine that forms a salt bridge with S2 or S3 aspartate or glutamate residues allow an alternate interpretation of the results of MTS mutation experiments, as the cavity resulting from the mutation could accommodate an MTS reagent. The position of protons in the voltage sensing domains (VSD) alters the conformation of the aromatic residues, thus affecting intracellular vs. extracellular access of MTS reagents to the cysteines in the mutant, rendering ambiguous the results of MTS experiments on R→C mutants. In further work on salt bridges, we have done quantum calculations (all DFT: B3LYP/6-311++G\*\*) on a

salt-bridge-like “ring”, in which a carboxylic acid and guanidinium (as in arginine) are separated by two water molecules, each connecting a carboxyl oxygen and a guanidinium nitrogen. Another complete set of calculations with a third, non-ring, water, showed how a small perturbation makes a large difference. Calculations in which one water was displaced by 1 Å and (separate calculation) by 3 Å, away from its ring position, (without reoptimization) demonstrate the effect of displacing one ring water on the charges of atoms of the other water, and on several other atoms, as well as on the bond orders, suggesting the ring shows resonance. For all cases, Natural Bond Order (NBO) calculations were also done. Separate calculations performed with the acid and base neutral, and ionized, indicate conformations of the ring depend on water molecule relations to the acid/base pair, as well as their charge state. Such rings may exist in proteins, including the VSD of ion channels.

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#### State Dependent Photo-Crosslinking of the IKS Channel Complex Demonstrates Movement of KCNE1 at Pre-Opening Membrane Potentials

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The slow delayed rectifier current (IKS) is a key repolarizing potassium current in the cardiac action potential. IKS is composed of KCNQ1 which forms the tetrameric voltage gated pore subunit and KCNE1, a single transmembrane domain accessory subunit, proposed to reside in the channel's exterior cleft. KCNE1 imposes a dramatic regulation on KCNQ1, significantly delaying opening compared to the unchaperoned channel. Here, we have investigated the dynamics of this interaction using the UV-crosslinking unnatural amino acid, p-benzoyl-L-phenyl alanine (pBpa). pBpa was genetically incorporated into KCNE1 at residue F57 in the transmembrane domain using the amber stop codon (TAG) suppression system. Characterization of the pBpa-incorporated channel complex revealed a 9 mV left shift in V<sub>0.5</sub> of activation compared to wild type. To evaluate the channel's activation pathway, cells were held for 2s at a range of non-activating potentials (−110 - −30 mV) followed by a 4s activation step to +60 mV. Increasing the holding potential progressively reduced activation time confirming multiple closed-states. Crosslinking was induced for each non-activating potential by repeatedly applying a 300 ms flash of UV light at the end of the 2s hold followed by a 4s activation step to +60 mV. Analysis of the change in peak current vs. cumulative UV-exposure revealed a rapid decrease compared to wild type channels indicating the permanent trapping of closed channels. The greatest rates of crosslinking were found at the most hyperpolarized holding potentials but no significant change in rate was observed above −70 mV, indicating that KCNE1 has moved outside the pBpa crosslinking radius as the channels progress through the activation pathway. This initial movement of KCNE1 suggests that inhibition of KCNQ1 occurs in a closed-state closer to the open-state in the activation pathway.

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#### Testing the Hydration Status of the Shaker-K Channel Voltage Sensor Domain with Sugars

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Voltage gated K-channels are formed by two well defined domains: the Pore Domain (PD), which is responsible for the K<sup>+</sup> ions conduction process, and the Voltage Sensor Domain (VSD), which sense the transmembrane potential due to the presence of several charged moieties. VSD moves upon activation such that ~4 net positive charges translocate across the membrane. The molecular details of such movement have been subject of intense controversy. We asked if part of the charges are hydrated at the resting and activated states, and if they change their hydration status during voltage activation. We measured the gating currents of a constitutively closed Shaker-V478W in macro patches of *Xenopus* oocytes in the presence of internal, external, or symmetric 2M Sucrose to reduce the water availability for eventual VSD hydration. Our results are consistent with the idea that some charged residues are hydrated when exposed to the cytosol at resting, dehydrate before translocation and rehydrate externally in the activated conformation. These suggest that water plays an important role stabilizing charged moieties in both, the resting and active conformation, revealing a novel role for water in the voltage sensing process.

ID-F is a MECESUP Fellow. We thank to Iniciativa Científica Milenio (P09-022-F) and Fondecyt 1120819.